Phosphorus-friendly transgenics

Transgenic animals engineered to express a bacterial enzyme that liberates phosphate from animal feed may provide a solution to a common form of environmental pollution.

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Environmental pollution attributable to intensive animal agriculture is increasingly recognized as a serious ecological problem[1]. In this issue, transgenic mice have been engineered to more efficiently metabolize phosphates from feed, thereby reducing the phosphate content of their excreta[2]. Once adapted to large animals, this approach could lower the phosphate content of manure from intensively reared livestock and potentially reduce the risk of phosphate contamination of water sources.

Inorganic phosphate is frequently added to animal feed to facilitate optimal growth. This is because phytate (inositol hexaphosphoric acid), a naturally occurring phosphate present in cereal grains, legumes, and oilseeds, etc., is resistant to absorption by animals. When excess phosphate leached from manure enters our waterways, it can stimulate algal blooms and eutrophication. Thus, the livestock industry is actively seeking ways of ensuring that the effluent from intensively reared animals contains less phosphate than at present.

One potential solution is to supplement animal feed with phosphorolytic enzymes that promote release of the mineral from plant material. The addition of phytase, a bacterial enzyme that releases phytate from animal feed, can eliminate the need for dietary phosphorus supplementation. But this approach is costly and the enzyme is subject to partial or complete inactivation during preparation and storage of the feed.

Golovan *et al.*[2] have taken a more direct approach by genetically manipulating phosphate metabolism in the animal itself. In their paper, they describe how a bacterial gene encoding phytase, inserted into the mouse genome by transgenic techniques and modified for expression in the animal's salivary glands, can significantly reduce the amount of phosphate excreted by a transgenic mouse compared with nontransgenic controls (Fig. 1). Bacterial phytase is secreted by the salivary glands of the transgenic mice, thus providing a stable and cost-free source of the enzyme. Golovan *et al.* report an 11% reduction in the fecal phosphorus content of their transgenic mice and suggest that this figure will be substantially higher in animals such as pigs, because mice probably recycle fecal phytate through coprophagy.

The paper represents the first step in applying to animal agriculture the concept of phosphorous pollution control via a transgene. It is a significant achievement to obtain correct tissue-specific expression of a transgene containing a bacterial coding sequence. Furthermore, the authors have shown that the bacterial enzyme can be produced constitutively in quantities that are more than sufficient to provide the animals' total phosphate requirement.

The next step in this line of research is clearly to repeat these studies in a farm animal, such as the pig, one of the prime industrial targets. Although conventional microinjection might well be used for this purpose, the recent demonstration of successful nuclear transfer in pigs by Betthauser *et al.*[3] probably provides a more reliable and economical approach for this next stage. The objective is surely worthwhile, because an animal that does not need phosphorus supplementation under intensive rearing conditions would be economically advantageous as well as much more environmentally acceptable.

The next phase of the work will not be without its challenges, however. Scientists will need to achieve the correct tissue specificity and appropriate levels of expression in the larger animal. It is not unknown for transgenes to function as predicted in transgenic mice but not in larger animals, and as a consequence, the suitability of the laboratory mouse as a model for transgene expression studies in larger animals is unclear. Furthermore, previous attempts to alter the digestive capabilities of larger animals by enzyme-encoding transgenes have not succeeded, despite their promise in laboratory mice[4].

In the present case, however, there is good reason to believe that the results could be duplicated in larger animals. This view is based on work reported a number of years ago on the ability of milk-protein gene promoters to direct expression of foreign proteins in the mammary glands of animals[5]. In those studies, the expression

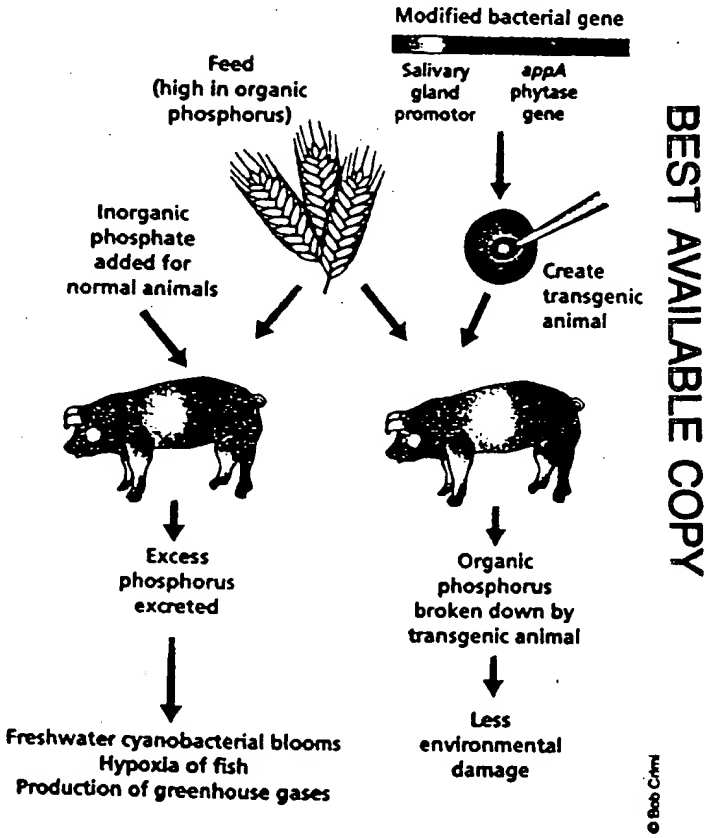

Figure 1. A transgenic approach to reducing environmental phosphate pollution. Expression of a bacterial phytase gene in the salivary gland allows the animal to digest organic phosphorous, removing the need for dietary supplementation with inorganic phosphate.

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found in mice was more often than not also found to occur in sheep, so that the mouse was an excellent model for the large-animal research. Because the salivary gland, like the mammary gland, is a secretory tissue, the promoter used by Golovan *et al.* in mice may well direct expression to the salivary glands of larger animals as predicted. Indeed, the researchers have apparently already produced transgenic pigs expressing salivary phytase.

The expression of bacterial genes in animals is an attractive use of transgenic technology to improve agricultural productivity[6], but it does present a number of potential problems. Paramount among these is the need to convince a skeptical public that the new genetic information is unlikely to be deleterious to human health. The genetic manipulation of plants destined to enter the food chain—either directly, as part of the human diet, or as feed for animals that are later consumed—has created a considerable divergence of opinion in society. One of the concerns frequently expressed by opponents of genetic manipulation technologies is the possibility that a novel protein present in the transgenic organism might possess allergenic properties. In the research under discussion, a bacterial protein is being secreted into the animal's digestive tract. The protein is certainly destined to be digested there, but it will still be necessary to demonstrate that the protein is not detectable in the parts of the animal carcass that are eaten. If the gene is expressed in tissues other than the salivary gland and produces phytase enzyme in edible parts of the animal, scientists will need to show that the bacterial protein is not itself a dangerous allergen for humans. This is a difficult task, but one that will be faced more and more frequently by scientists wishing to transfer and express foreign genes in domestic animals.

The paper by Golovan *et al.* promises to be a significant step toward improved agricultural productivity and enhanced environmental protection using these powerful transgenic techniques.

It has been almost 20 years since the pioneering paper of Palmiter *et al.*[7] demonstrated the use of recombinant DNA to alter an animal's phenotype, and in doing so, proposed that the technology held great promise for improving animal agriculture. Although major advances have been made since then in producing modified proteins in the mammary gland of transgenic domestic animals, little has been gained in improving animal rearing on the farm. The paper by Golovan *et al.* promises to be a significant step toward improved agricultural productivity and enhanced environmental protection using these powerful techniques.

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